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The Fate of Imidacloprid in Tobacco Smoke of Cigarettes Made from Imidacloprid-Treated Tobacco

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Abstract: The residues and metabolites of radiolabelled imidacloprid [1-(6-chloropyridin-3-ylmethyl)-*N*-nitroimidazolidin-2-ylideneamine], formulated as a wettable powder containing 250 g kg^{-1} active ingredient diluted with water and administered to tobacco plants, were studied in sidestream and mainstream smoke, in the ash and butts after smoking cigarettes. An almost complete recovery of radioactivity (93·5%) was achieved. The highest amounts of radioactivity were found in the butts and sidestream smoke. The two dominant compounds identified after smoking were unchanged parent compound and carbon dioxide. A total of 76% of the recovered radioactivity was identified. © 1998 SCI.

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Key words: imidacloprid; metabolism; cigarettes; smoke; chloronicotinyl; radiolabelling

1 INTRODUCTION

Imidacloprid [1-(6-chloropyridin-3-ylmethyl)-*N*-nitroimidazolidin-2-ylideneamine] is a relatively new, highly effective systemic and contact insecticide exhibiting low mammalian toxicity. It is currently the most important representative of a new type of insecticide, namely the chloronicotinyl class of compounds. These compounds interfere with the signal transmission in the insect nerve system acting at a nicotinic acetylcholine receptor (nAChR).[1–3] Imidacloprid controls a wide range of insect pests including aphids, whiteflies, thrips, scales, leafhoppers and planthoppers.

Imidacloprid has been registered for use in more than 70 countries worldwide and has a favourable safety and toxicology profile. Its metabolism in a wide variety of crops, such as tomatoes, potatoes, apples, corn, cotton, rice and others, has already been investigated and reported for registration purposes.[4]

In order to check that pesticides have no adverse effect on the taste of cigarettes from the smoker's point of view, flavour studies on cigarettes produced from pesticide-treated tobacco have to be conducted by cigarette manufacturers. Prior to initiating these studies, it is prudent to ensure that undesirable pesticide-derived pyrolysis or combustion products are not transferred in relevant amounts in smoke. Within that framework, the objective of this study was to investigate the fate of imidacloprid after soil drench and spray application to tobacco plants with regard to the transfer of imidacloprid, its metabolites and pyrolysis products into the smoke of cigarettes made from leaves of these imidacloprid-treated plants.

2 MATERIAL AND METHODS

2.1 Test chemicals and reference compounds

[pyridinyl-^{14}C-methyl]imidacloprid with a specific radioactivity of 1·15 MBq mg^{-1} and a radiochemical

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purity of 99% was supplied by Bayer AG, Elberfeld, Germany and, prior to application, it was formulated as a wettable powder containing 250 g kg^{-1} active ingredient (WP 25). Additional nonradiolabelled reference compounds supplied by Bayer AG, Monheim, Germany were imidacloprid, the guanidine **2** (1-(6-chloropyridin-3-ylmethyl)imidazolidin-2-ylideneamine), the urea **3** (1-(6-chloropyridin-3-ylmethyl)imidazolidin-2-one), the olefin **4** (1-(6-chloropyridin-3-ylmethyl)-*N*-nitroimidazolin-2-ylideneamine), the nitrosimine **5** (1-(6-chloropyridin-3-ylmethyl)-*N*-nitrosoimidazolidin-2-ylideneamine) and the 4-hydroxy compound **6** (1-(6-chloropyridin-3-ylmethyl)-2-(nitroimino)imidazolidin-4-ol) (Fig. 1).

2.2 Application

One soil application and three spray applications were made to a total of 11 tobacco plants. Owing to legal constraints on the use of radioactivity within the glasshouse facility, two separate experiments were conducted in parallel. At harvest, all sampled leaves from both experiments were combined and treated as one sample. The soil applications were made on 16 September, the first spray applications on 26 October, the second on 2 November and the third on 8 November, all in 1993. The stability of the parent compound in the application solutions was confirmed by thin-layer chromatography (TLC) before and after each application, both for the soil application and the three spray applications.

2.2.1 *Soil application*

The amount applied to the soil around each of the 11 tobacco plants individually grown in 15-litre pots was 80 mg of WP 25 prepared from radiolabelled imidacloprid in 120 ml water, i.e. 20 mg active ingredient (AI), which is equivalent to 23·0 MBq.

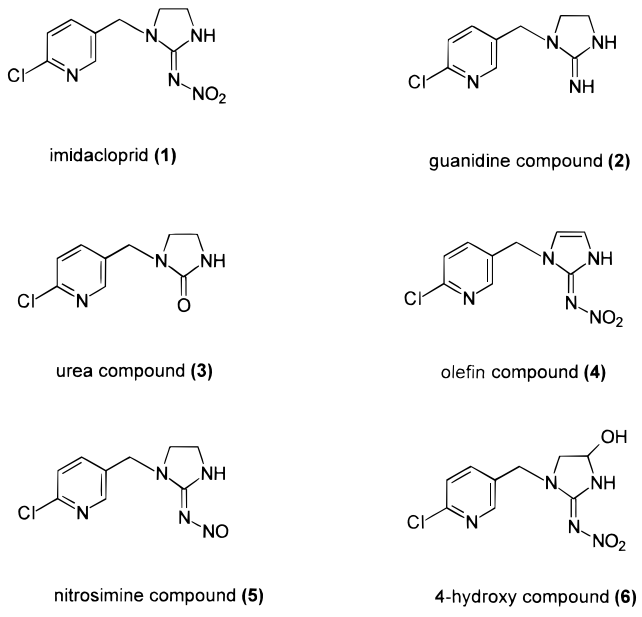

Fig. 1. Structures of parent and reference compounds.

2.2.2 *Spray application*

In order to ensure that, at the time of harvest, all treated leaves were, as far as possible, at the same maturity state, the four leaves at the base of the plants were not treated. The eight leaves immediately above these were all individually sprayed using a retouch microsprayer (EFBE airbrush, Boldt, Hannover, Germany). The leaves above these eight were not treated.

The same procedure was followed for each application: water (96 ml) was added to 134·4 mg of the WP 25 prepared from radiolabelled imidacloprid. An aliquot (1 ml) of the resulting suspension was applied to each individual leaf. This was equivalent to spraying 0·35 mg AI per leaf. For the three applications, this yielded a total amount applied per leaf of 1·05 mg AI, and per plant 8·4 mg AI, which was equivalent to 9·66 MBq.

The total amount applied to the soil and the 11 plants amounted to 1249·6 mg WP 25, 312·4 mg AI which was equivalent to 359·26 MBq.

2.3 Sampling and conditioning

A total of 88 leaves was harvested two weeks (22 November 93) after the last spray application, of which 24 leaves (1380 g fresh weight) at the same stage of maturity were selected for air-curing and preparation of cigarettes for the smoking experiments.

2.4 Preparation of cut tobacco and cigarettes

Lamina of air-cured (four months, 22(±1)°C, 50(±5)% RH, air flow 1 m s^{-1}) and conditioned (approx. three days, 22(±1)°C, 85% RH) tobacco leaves were cut with a modified tissue chopper (McIllwain tissue chopper, The Mickle Laboratory Engineering Company, Gomshall, UK) at 35 cuts per inch.

Commercially available filter sleeves were filled with approximately 0·8 g of the cut tobacco and the filter cigarettes conditioned (approximately two days, 22(±1)°C, 65% RH). The tobacco content of each single cigarette was determined by weight. Before loading the smoking machine, the filters were cut off, resulting in filterless cigarettes of approx. 68 mm length.

2.5 Smoking

Each of 10 smoking experiments was performed using four cigarettes which were consecutively smoked on a single-port total-recovery smoking machine (Philip Morris, Richmond, VA, USA) in as close conformity as possible with ISO 3402,[5] ISO 3308[6] and ISO 4387.[7] After lighting with a halogen spot lamp, the cigarettes

were smoked down to a butt length of 23 mm, the puff volume being 35 ml. The cigarettes were puffed every minute.

The smoking machine worked essentially as previously described.[8] The cigarette was placed in the cigarette holder of a glass smoking chamber which was supplied with air or nitrogen from pressurized cylinders. In each experiment, the flow rates for mainstream smoke (MS) and sidestream smoke (SS) were adjusted with the aid of flowmeters to 1050 and 1500 ml min^{-1}, respectively, before lighting the first cigarette, and the flows were controlled by computer-driven solenoid valves. During the 2-s puff duration, the MS path was opened and the SS path closed. The MS passed through a glass-fibre filter (Cambridge Filter Pads, 44 mm diameter, Filtrona, Reinbek, Germany) and the resulting gas/vapour phase of the MS through various traps as described in Section 2.6.2. For the time period between two puffs (58 s) the MS path was closed and the air passed around the cigarette collecting the SS from the smoking chamber into the SS path of the smoking machine. The SS passed through an identical series of filter and traps to the MS. Extensive flushing of the gas/vapour duct of the MS path between the puffs was performed with nitrogen and of the SS path during puffing with air. After the last puff of each cigarette, the cigarette was extinguished by flushing the smoking chamber with nitrogen, after which the whole system was extensively flushed with nitrogen for 4 min.

2.6 Determination of total radioactive residue (TRR) and recovered radioactivity (RR)

Radioactivity in liquid samples was quantified by liquid scintillation counting (LSC) (Tri-Carb 2550 TR, Canberra-Packard, Frankfurt a.M., Germany). Radioactivity in solid samples was quantified after combustion of the samples or aliquots thereof in a sample oxidizer (OX 500, Harvey Instruments *via* Zinsser Analytic, Frankfurt a.M., Germany), and the resulting carbon dioxide was trapped in Oxysolve C-400 (Zinsser Analytic, Frankfurt a.M., Germany). The total radioactive residues (TRR) were calculated on a mg imidacloprid-equivalent per kg tobacco basis.

The recovered radioactivity (RR) was calculated as the sum of the total radioactivity found in the butt, in the ash, in the trapped smoke fractions of both MS and SS, and that remaining in the apparatus. This value was normalized to the total radioactivity in all cigarettes, calculated from the amount of tobacco in these cigarettes and the TRR determined in the cigarette tobacco (see Section 2.6.1).

2.6.1 Determination of TRR of the tobacco in the cigarettes

All the tobacco in each of five cigarettes was removed from the paper and individually homogenized in a blender. The radioactivity in three aliquots (approximately 50 mg each) of the homogenized tobacco of each cigarette was determined after sample oxidation.

2.6.2 Determination of TRR in butts, ash and smoke fractions

The four butts (including paper) of each individual experiment were combined, weighed and homogenized. The ash from the four cigarettes in each individual experiment was combined, weighed and thoroughly mixed. The TRR in butts and ash was determined after sample oxidation.

The filter pads containing the particle phases of the MS and SS of the four cigarettes in each experiment were removed from the respective filter holders, placed into glass bottles containing acetone (20 ml), and sonicated for 20 min. Each filter was washed with acetone (3×10 ml). The acetone suspensions of the particle phases were combined with the respective washing solutions in 50-ml volumetric flasks and filled up to the mark with acetone. The radioactivity in the acetone solution was determined by LSC in Quicksafe N (Zinsser Analytic, Frankfurt a.M., Germany). The radioactivity in the air-dried extracted filters was determined after sample oxidation.

The traps for polar compounds in the gas/vapour phase of MS and SS contained dilute hydrochloric acid solution (170 ml, 4 mM). After smoking the four cigarettes of each experiment, the radioactivity in the traps was determined by LSC in Aquasafe 300 Plus (Zinsser Analytic, Frankfurt a.M., Germany).

The traps for carbon monoxide in the gas/vapour phase of MS and SS contained copper (I) chloride (0·8 M) dissolved in hydrochloric acid (170 ml; 4 M). After smoking the four cigarettes of each experiment, the radioactivity in the traps was determined by LSC in a gel formed from sample aliquots, Quicksafe A (Zinsser Analytic, Frankfurt a.M., Germany), and water.

The traps for apolar compounds in the gas/vapour phase of MS and SS contained isooctane (170 ml). After smoking the four cigarettes of each experiment, the radioactivity was determined by LSC in Quicksafe N (Zinsser Analytic, Frankfurt a.M., Germany).

The traps for carbon dioxide in the gas/vapour phase of MS and SS contained aqueous sodium hydroxide (170 ml, 1 M). After smoking the four cigarettes of each experiment, the radioactivity was determined by LSC in Aquasafe 300 Plus (Zinsser Analytic, Frankfurt a.M., Germany). In order to confirm that the radioactivity in the sodium hydroxide traps originated only from trapped carbon dioxide, representative aliquots of 10 ml were taken and any trapped carbon dioxide was precipitated as barium carbonate by adding aqueous barium chloride (20 ml; 0·5 M) containing ammonium chloride (0·4 M). The precipitate was filtered off under suction. The precipitate on the filter was washed with

barium chloride/ammonium chloride solution (10 ml). This washing solution was combined with the filtrate, thus ensuring that the precipitation was complete. Radioactivity in the filtrate was determined by LSC.

At the end of experiments 5 and 10, the total-recovery smoking machine was extensively washed. Washing was performed separately for the MS and SS paths. All ducts, tubings, filter holders, the smoking chamber as well as the trapping bottles were washed using methanol, except the trapping bottles for polar compounds, carbon monoxide and carbon dioxide, which were washed with water. The respective washing solutions obtained after experiment 5 were combined with those after experiment 10. It was considered that the methanolic washing solution represented particle phase deposits, whereas the aqueous washing solution represented deposits from the gas/vapour phase. For each of the four combined washing solutions, radioactivity was determined by LSC. It was assumed that one-tenth of this radioactivity remained in the apparatus during one experiment; this activity was taken into account when calculating the recovery of each single experiment.

2.7 Quantitation of imidacloprid, its metabolites and its pyrolysis products by two-dimensional TLC

The homogenized tobacco from the butts of the first five experiments was extensively extracted by maceration in four successive steps: with methanol + water (20 ml; 1 + 1, by volume), with methanol (15 ml), and with dichloromethane (2 × 15 ml). After the last extraction step, the suspension was filtered under suction and the extracted butt tobacco was washed on the filter with dichloromethane (5 ml) and methanol (5 ml). The decanted supernatants and wash fluids were combined. The biphasic extraction fluid was filled up with methanol until a single phase was obtained. The TRR was determined in the extract as well as in the air-dried extracted filter. The extract was used for TLC analysis.

The acetone extracts of the MS particle filters of the first five experiments were combined and the pooled solution analyzed by TLC. This was similarly repeated for the SS particle filter. The methanol washing of the SS path of the apparatus was used as it was.

Two-dimensional TLC was performed using silica gel plates (Kieselgel 60 F_{254}, thickness 0·25 mm, 20 × 20 cm; Merck, Darmstadt, Germany) after spot application. The solvent systems were as follows: A: 1st dimension: chloroform + methanol + acetic acid + water (65 + 25 + 3·5 + 3·5, by volume), 2nd dimension: butan-1-ol + acetic acid + water (80 + 20 + 20, by volume); B: 1st dimension: ethyl acetate + toluene + methanol + acetic acid (80 + 20 + 20 + 1, by volume), 2nd dimension: ethyl acetate + propan-2-ol + water(65 + 23 + 12, by volume). The plates were carefully dried between developments.

The evaluation of the TLC plates was performed after exposure for three days to a phosphor screen by radiometric scanning (BAS 2000 Bioimaging Analyser, Fuji *via* Raytest, Straubenhardt, Germany). The quantitative distribution of metabolites was determined by using the 'Tina' software, version 2.08a (Raytest). Co-chromatographed reference compounds were visualized on the TLC plates by UV light at 254 nm.

3 RESULTS AND DISCUSSION

3.1 Tobacco and cigarettes

The TRR in the air-cured tobacco of the cigarettes was 126·4(±7·5) mg imidacloprid equivalents kg^{-1} (mean ± SD). The cigarettes used in the experiments had a tobacco weight of 0·782(±0·065) g (mean ± SD). A mean number of 8·5 puffs (SD: 1·2) was obtained by smoking the cigarettes following as closely as possible the pertinent ISO standards.

3.2 Radioactivity in butt, ash and smoke fractions

From the total radioactivity in the air-cured tobacco, 93·5% was recovered after cigarette smoking. The normalized results for the individual smoke fractions are given in Table 1. Of the total RR (i.e. 100%), the butt contained 39·3% and the ash 5·0%. Radioactivity in the ash represents most probably SS components which were adsorbed on the large surface of the ash. A total of 34·6% of RR was identified as carbon dioxide and 0·5% as carbon monoxide. From the remaining RR, 5·3% was found in the MS (0·2% in gas/vapour phase and 5·1% in particle phase) and 15·3% in the SS (7·0% in gas/vapour phase and 8·3% in particle phase).

The TRR in the MS was 10·2 mg kg^{-1} (6·5 mg kg^{-1} in particle and 3·7 mg kg^{-1} in gas/vapour phase), 60·3 mg kg^{-1} in the SS (10·5 mg kg^{-1} in particle and 49·8 mg kg^{-1} in gas/vapour phase), 49·6 mg kg^{-1} in the butt and 6·3 mg kg^{-1} in the ash.

3.3 Quantitation and identification of imidacloprid, its metabolites and pyrolysis products

3.3.1 Butts

The butts were extracted with an efficiency in recovery of radioactivity of 95·6% (TRR: 47·4 mg kg^{-1}); 4·4% of the radioactivity (TRR: 2·2 mg kg^{-1}) remained unextracted. The major compound identified by two-dimensional TLC was unchanged imidacloprid (Fig. 2), accounting for 72% of the radioactivity in the butt (Table 2). Furthermore, compounds **2**, **3**, **4**, **5** and **6** were also identified by two-dimensional TLC in the butt extract, but in minor amounts. These results reflected

TABLE 1
Distribution of Radioactivity in the Different Smoke Fractions Obtained after Smoking Cigarettes Prepared from Imidacloprid-Treated Tobacco

Fraction	*Recovered radioactivity*[a] (% *of total*)
Mainstream smoke	
Particle phase	
Filter extract	4·6 (±1·5)
Not-extractable	0·2 (±0·2)
Wash	0·3[b]
Subtotal	5·1 (±1·6)
Gas/vapour phase traps	
Dilute acid	0·2[c]
Isooctane	< 0·05[c]
CuCl/HCl (for CO)	0·1[c]
NaOH (for CO_2)	2·7 (±0·4)
Wash	< 0·05[b]
Subtotal	2·9 (±0·4)
Sum	8·0 (±2·0)
Sidestream smoke	
Particle phase	
Filter extract	5·8 (±0·9)
Not-extractable	0·3 (±0·1)
Wash	2·2[b]
Subtotal	8·3 (±1·0)
Gas/vapour phase traps	
Dilute acid	6·7 (±0·6)
Isooctane	0·2[c]
CuCl/HCl (for CO)	0·4 (±0·1)
NaOH (for CO_2)	31·9 (±4·1)
Wash	<0·05[b]
Subtotal	39·4 (±4·8)
Sum	47·7 (±5·6)
Butts	39·3 (±6·5)
Ash	5·0 (±1·3)
Total	100·0 (±6·7)

[a] Normalized results; mean(±SD) of 10 experiments. Recovered radioactivity (93·5%) is based on radioactivity in cigarettes. Total radioactive residue is equal to 126·4 mg imidacloprid-equivalents kg^{-1} tobacco. Deviations in the last digit of sums may arise from rounding.
[b] n = 1
[c] SD < 0·05

those obtained in other plant metabolism studies for registration, a summary of which has been published previously.[4]

3.3.2 *Mainstream smoke*

The particle filters in the MS path of the smoking machine were extensively extracted with a mean extraction efficiency of radioactivity of 95·3% (TRR: 5·8 mg kg^{-1}); 4·7% of the radioactivity (TRR: 0·3 mg kg^{-1}) remained unextracted. The radioactivity recovered by washing the MS ducts and filter holder of the apparatus accounted for 0·3% (TRR: 0·4 mg kg^{-1}).

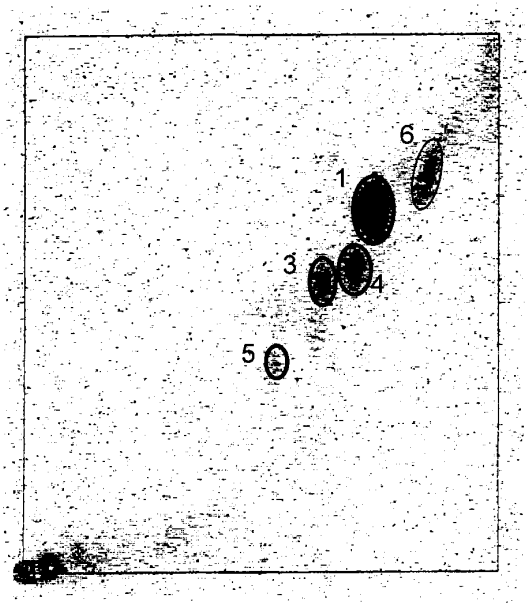

Fig. 2. Two-dimensional TLC, radiometric evaluation of the butt extract. TLC was performed using solvent system B. Numbers refer to compounds shown in Fig. 1.

This fraction was not further analyzed. The major compounds identified by two-dimensional TLC were the urea compound **3** and unchanged imidacloprid (31 and 23% of the radioactivity, respectively, see Fig. 3 and Table 2).

No further analysis of polar compounds (trapped in dilute acid solution, 0·2% of RR) and of apolar compounds (trapped in isooctane, 0·04% of RR) was performed. The radioactivity trapped in copper(I)chloride/hydrochloric acid (0·06% of RR) was assumed to be carbon monoxide, as this reagent is considered to be specific for trapping that compound.[9] The

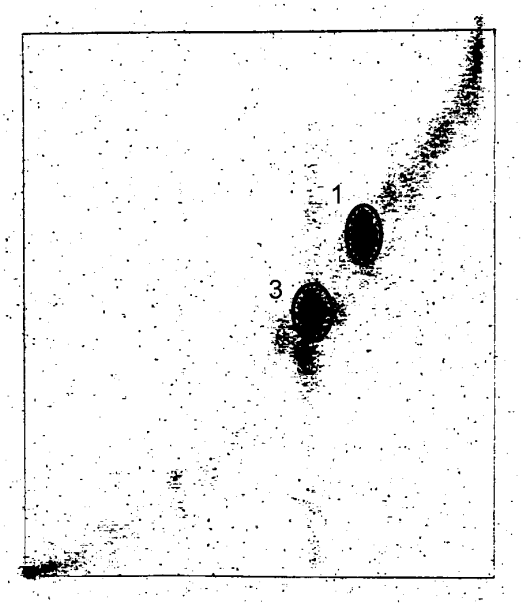

Fig. 3. Two-dimensional TLC, radiometric evaluation of the MS particle filter extract. TLC was performed using solvent system B. Numbers refer to compounds shown in Fig. 1.

TABLE 2
Distribution of Imidacloprid, Its Metabolites and Pyrolysis Products in the Different Smoke Fractions

	Recovered radioactivity (% of total)								
	MS			*SS*					
	Particle phase		*Gas/Vapour phase*	*Particle phase*		*Gas/Vapour phase*			
Compound	*Filter*	*Wash*		*Filter*	*Wash*		*Butt*	*Ash*	*Sum*[b]
Imidacloprid (**1**)	1·2	—	—	0·8	0·2	—	28·3	—	30·4
Guanidine compound (**2**)	n.d.[a]	—	—	n.d.	n.d.	—	1·3	—	1·3
Urea compound (**3**)	1·6	—	—	2·0	0·3	—	1·2	—	5·0
Olefin compound (**4**)	n.d.	—	—	n.d.	n.d.	—	2·2	—	2·2
Nitrosimine compound (**5**)	n.d.	—	—	n.d.	n.d.	—	0·3	—	0·3
4-Hydroxy compound (**6**)	n.d.	—	—	n.d.	n.d.	—	1·6	—	1·6
CO	—	—	0·1	—	—	0·4	—	—	0·5
CO_2	—	—	2·7	—	—	31·9	—	—	34·6
Unidentified	2·0	0·3	0·2	3·3	1·7	7·0	4·3	5·0	23·8
Total[b]	4·8	0·3	3·0	6·1	2·2	39·3	39·2	5·0	100·0

[a] n.d.: not detected.
[b] Deviations in the last digit of sums may arise due to rounding.

radioactivity trapped in sodium hydroxide (2·7% of RR) was precipitated with barium chloride to an extent of 98·8%, thus indicating that almost all of the trapped radioactivity was carbon dioxide.

3.3.3 Sidestream smoke

The particle filters in the SS path of the smoking machine were extensively extracted with a mean extraction efficiency of radioactivity of 94·6% (TRR: 7·3 mg kg^{-1}); 5·4% of radioactivity (TRR: 0·4 mg kg^{-1}) remained unextracted. The major compounds identified by two-dimensional TLC were the urea compound, **3**, and unchanged imidacloprid (24 and 9% of the total radioactivity in the SS particle phase, respectively, Table 2). The radioactivity recovered by washing off the condensate from the smoking chamber, the ducts to the filter, and the filter holder of the SS was 2·2% of RR. In this wash fraction, only unchanged imidacloprid (8%) and compound **3** (14%), representing 2 and 4%, respectively, of the total radioactivity in the SS particle phase (Table 2), was identified by two-dimensional TLC.

The trapping solution for polar compounds (dilute acid solution) contained 6·7% of the RR. Analysis of this fraction by two-dimensional TLC yielded only diffuse radioactivity (not in peaks; data not shown) and was therefore not investigated further. No further analysis of apolar compounds (trapped in isooctane, 0·2% of RR) was performed due to the low amount of radioactivity. The radioactivity trapped in copper(I)chloride/hydrochloric acid (0·4% of RR) was assumed to be carbon monoxide. A major portion of the radioactivity was trapped in sodium hydroxide (32% of RR). By precipitation with barium chloride it was shown that all of the trapped compound (99.8%) was carbon dioxide.

4 CONCLUSIONS

Using the smoking apparatus described in this publication, an almost complete recovery of radioactivity (93·5%) was achieved. The amount of carbon dioxide present in the SS represented almost 60% of the radioactivity found in total in MS and SS and was by far the main component of the smoke fractions. This indicated that imidacloprid was primarily degraded to carbon dioxide during smoking. The only other components identified in the smoke fractions were unchanged parent compound, the urea compound, **3**, and small amounts of carbon monoxide. Further metabolites identified in trace amounts in the butts have also been previously identified in a variety of plant metabolic studies. In total, 76% of the recovered radioactivity (TRR: 96.1 mg kg^{-1}) was identified.

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